Cholesterol 25-hydroxylase activity of CYP3A

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Abstract To date, many studies have been conducted using 25-hydroxycholesterol, which is a potent regulator of lipid metabolism. However, the origins of this oxysterol have not been entirely elucidated. Cholesterol 25-hydroxylase is one of the enzymes responsible for the metabolism of 25-hydroxycholesterol, but the expression of this enzyme is very low in humans. This oxysterol is also synthesized by sterol 27-hydroxylase (CYP27A1) and cholesterol 24-hydroxylase (CYP46A1), but it is only a minor product of these enzymes. We now report that CYP3A synthesizes a significant amount of 25-hydroxycholesterol and may participate in the regulation of lipid metabolism. Induction of CYP3A by pregnenolone-16α-carbonitrile caused the accumulation of 25-hydroxycholesterol in a cell line derived from mouse liver. Furthermore, treatment of the cells with troleandomycin, a specific inhibitor of CYP3A, significantly reduced cellular 25-hydroxycholesterol concentrations. In cells that overexpressed human recombinant CYP3A4, the activity of cholesterol 25-hydroxylation was found to be higher than that of cholesterol 4β-hydroxylation, a known marker activity of CYP3A4. In addition, 25-hydroxycholesterol concentrations in normal human sera correlated positively with the levels of 4β-hydroxycholesterol (r = 0.650, P < 0.0001, n = 78), but did not significantly correlate with the levels of 27-hydroxycholesterol or 24S-hydroxycholesterol. These results demonstrate the significance of CYP3A on the production of 25-hydroxycholesterol. — Honda, A., T. Miyazaki, T. Ikegami, J. Iwamoto, T. Maeda, T. Hirayama, Y. Saito, T. Teramoto, and Y. Matsuzaki. Cholesterol 25-hydroxylase activity of CYP3A. J. Lipid Res. 2011. 52: 1509–1516.

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Oxysterols are physiological regulators of cellular cholesterol homeostasis (1). They downregulate HMG-CoA reductase (2–4), the rate-limiting enzyme in the cholesterol biosynthetic pathway, by blocking processing of the sterol-regulatory element binding protein (SREBP) by inducing binding of SREBP cleavage-activating protein to a protein called Insig (insulin-induced gene) (5, 6). Furthermore, there is growing evidence that certain oxysterols may accelerate ubiquitination and degradation of HMG-CoA reductase protein (1, 7). On the other hand, oxysterols are endogenous ligands of the nuclear receptor liver X receptor α (LXRα; NR1H3) (8–10), which modulates immune responses and regulates various metabolic pathways, including cholesterol, bile acids, FAs, and glucose (11, 12).

In in vitro experiments, 25-hydroxycholesterol is widely used as a potent inhibitor of HMG-CoA reductase or as a ligand of LXRα, but the origins of this oxysterol are not entirely clear. Enzymatic production of 25-hydroxycholesterol has been reported by microsomal cholesterol 25-hydroxylase (CH25H) (13), and the activation of Toll-like receptors, a class of proteins that play a key role in the innate immune system, markedly induces CH25H and increases 25-hydroxycholesterol concentrations in mice macrophages and sera (14, 15). In comparison with mice, however, expression of CH25H has been reported to be very low in human tissues (13). Other enzymes involved in the production of 25-hydroxycholesterol are mitochondrial sterol 27-hydroxylase (CYP27A1) (16, 17) and brain-specific microsomal cholesterol 24S-hydroxylase (CYP46A1) (18). In addition, nonenzymatic generation of 25-hydroxycholesterol by autoxidation of cholesterol has also been described (19).

Previously, we measured hepatic concentrations of intermediates in bile acid synthesis in Cyp27−/− mice (20). In this series of analyses, we unexpectedly found that microsomal concentrations of 25-hydroxycholesterol were

Abbreviations: CH25H, cholesterol 25-hydroxylase; CTX, cerebrotendinous xanthomatosis; CYP27A1, sterol 27-hydroxylase; CYP46A1, cholesterol 24S-hydroxylase; Insig, insulin-induced gene; LXR, liver X receptor; PCN, pregnenolone-16α-carbonitrile; SREBP, sterol-regulatory element binding protein; SRM, selected reaction monitoring.

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significantly elevated in Cyp27<sup>−/−</sup> mice (unpublished observation). This might be caused by reduced metabolism of 25-hydroxycholesterol due to inhibition of 27-hydroxylation. However, it was also possible that 25-hydroxylation of cholesterol was stimulated by enzyme upregulation in the Cyp27<sup>−/−</sup> mice. We speculated that CYP3A was the enzyme that exhibited high cholesterol 25-hydroxylation activity because CYP3A was markedly upregulated in Cyp27<sup>−/−</sup> mice, and this enzyme was known to catalyze a similar reaction, i.e., 25-hydroxylation of 5β-cholestan-3α,7α,12α-triol (21).

The CYP3A subfamily consists of monoxygenases that catalyze many reactions involved in the metabolism of xenobiotics, steroid hormones, and bile acids (22). Cholesterol is also one of the substrates for CYP3A and is believed to be mainly metabolized to 4β-hydroxycholesterol (23, 24). The present study was undertaken to prove that CYP3A catalyzes not only 4β-hydroxylation but also 25-hydroxylation of cholesterol and to show the possibility that 25-hydroxycholesterol in normal human serum originates from CYP3A4.

**MATERIALS AND METHODS**

**Chemicals**

Pregnenolone-16α-carbonitrile (PCN) and troleandomycin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Cholesterol and desmosterol were obtained from Steraloids, Inc. (Newport, RI), and cholesterol was used as substrate for the enzyme assay after purification with disposable silica cartridge columns (25) to remove contaminated oxytosters. Additional reagents and solvents were of analytical grade.

**Cell culture**

AML12 cells, a differentiated, nontransformed hepatocyte cell line that was derived from transforming growth factor α-overexpressing transgenic mice (26) were purchased from American Type Culture Collection (Manassas, VA). Cells were seeded in 6-well plates and cultured in a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F12 medium (Invitrogen Japan KK; Tokyo, Japan) supplemented with 0.005 mg/ml insulin, 0.005 mg/ml transferrin, 5 ng/ml selenium, 40 ng/ml dexamethasone, and 10% FBS. When the cells were subconfluent, the medium was replaced with fresh medium containing PCN, troleandomycin, or desmosterol dissolved in 1% ethanol. Although 1% ethanol in the medium had no detectable effects on cell growth, the same concentration of ethanol was also added to the control wells. Cells were incubated at 37°C in a humidified incubator containing 5% CO<sub>2</sub> and 95% air.

**RNA measurements**

Total RNA was extracted from the cells using an AllPrep RNA/protein kit (QIAGEN KK; Tokyo, Japan). Reverse transcription was performed on 1 µg of total RNA using a first-strand cDNA synthesis kit for RT-PCR (Roche Diagnostics; Mannheim, Germany). Real-time quantitative PCR was performed on cDNA aliquots with FastStart DNA Master SYBR Green I and a LightCy- cler (Roche). The sequences of the oligonucleotide primer pairs used to amplify mouse mRNAs are 5′-GGGAGCATTGATCTCTTACTG-3′ and 5′-AAGACACTCTTGGAGGAGAC-3′ for Cyp3a11 (NM_007818), 5′-ACACCTACTTTGAAGACCCAT-3′ and 5′-TGCAACTTCTACCTCCAT-3′ for Cyp46a1 (NM_0010010), 5′-CTTCCTGCTGACCAATGAGT-3′ and 5′-AGGTTCCTTGGCAGGGCCTGAT-3′ for Cyp27a1 (NM_024264), 5′-CCAAGTCTCATAACGTCCTCA-3′ and 5′-CAGGGAAAGGGCAGAC-3′ for Ch25h (NM_009890) and 5′-CCTGTATGCTCGGTGCTGA-3′, and 5′-CCATGCTCTTGGCAAGTC-3′ for β-actin (X03672).

**Determination of sterol concentrations**

Sterol concentrations in cell homogenate and serum were measured using our previously described HPLC-ELSI-MS/MS method (27, 28). In brief, 5 µl aliquots of serum or cell homogenate (approximately 1 × 10<sup>6</sup> cells) were incubated with stable isotope-labeled oxytoster as internal standards in 1 N ethanolic KOH at 37°C for 1 h. Sterols were extracted with n-hexane, derivatized to picolinyl esters, and analyzed by HPLC-ELSI-MS/MS. Conventional derivatization was conducted at 80°C for 60 min, but room temperature for 30 min was chosen for the specific monopicolinol ester formation of 25-hydroxycholesterol. Monopicolinyl 25-hydroxycholesterol exhibited [M+Na+CH<sub>3</sub>CN]<sup>+</sup> ion as the base peak, and [picolinic acid (C<sub>6</sub>H<sub>5</sub>NO<sub>2</sub>)+Na]<sup>+</sup> ion was observed as the most-abundant product ion under various levels of collision energy. Therefore, m/z 571 → 146 (25 V) and m/z 574 → 146 (25 V) were used as the monitoring ions and optimal
served in the PCN-treated cells, but the absolute mRNA

Marked upregulation of

were infected with a baculovirus containing the cDNA for rabbit

was evaluated using the Student's two-tailed
cance of differences between the results in the different groups

was tested by calculating Pearson's correlation coeffi cient,

all analyses, signifi cance was accepted at the level of

Enzyme assay

Microsomes (baculosomes) prepared from insect cells that

were infected with a baculovirus containing the cDNA for rabbit
cytochrome P450 reductase and human CYP1A2, CYP2C9,
CYP2D6, or CYP3A4 were purchased from Invitrogen. The mi-
crosomes (10 pmol of P450) were incubated for 30 min at 37°C
with various amounts of cholesterol (dissolved in 12 μl of a 33%
aqueous solution of 2-hydroxypropyl-β-cyclodextrin), NADPH
(1.2 mM), glucose-6-phosphate (3.6 mM), 2 U glucose-6-phos-
phate dehydrogenase, and 100 mM potassium phosphate buffer
(pH 7.4) containing 0.1 mM EDTA in a total volume of 0.5 ml.
The incubation was stopped by the addition of 1 ml ethanol. Af-
ter the addition of the internal standards and 5 μg butylic
hydroxytoluene to the mixture, oxysterols were extracted twice
with 2 ml n-hexane, derivatized to picolinyl esters, and analyzed
by HPLC-ESI-MS/MS, as described above. To exclude the possible
effects of contaminated oxysterols in substrate (cholesterol) and
cholesterol autoxidation, incubations without adding NADPH

generating system were conducted simultaneously, as a control,

and the data were subtracted from those obtained using com-
plete assay mixtures. An assay using boiled CYP3A4 was also con-
ducted to exclude the direct effects of the NADPH generating

system on cholesterol oxidation.

Statistics

Data are expressed as the mean ± SD. The statistical signifi-
cance of differences between the results in the different groups
was evaluated using the Student's two-tailed t-test. Correlation
was tested by calculating Pearson's correlation coeffi cient, r. For
all analyses, significance was accepted at the level of P < 0.05.

RESULTS

The effects of PCN, troleandomycin, and desmosterol
on sterol concentrations in AML12 cells are shown in
Fig. 1. The concentrations of 4β-hydroxycholesterol, 25-
hydroxycholesterol, and 22R-hydroxycholesterol were sig-
nifi cantly increased by treatment with PCN, a classical in-
ducer of CYP3A by the activation of pregnane X recep-
tor (NR1I2) (22). In contrast, these oxysterol concentra-
tions were significantly decreased by treatment with
troleandomycin, a specific inhibitor of CYP3A activity (30).
Furthermore, the increase of 25-hydroxycholesterol by
PCN treatment was not suppressed by the addition of des-
mosterol, a potent inhibitor of CH25H (13). On the other
hand, significant increase by PCN was not observed re-
garding the other oxysterol concentrations.

The effects of PCN, troleandomycin, and desmosterol
on mRNA expressions of Cyp3a11, Ch25h, Cyp46a1, and
Cyp27a1 in AML12 cells are shown in Fig. 2. Treatment
with PCN signifi cantly upregulated Cyp3a11 expression.
Marked upregulation of Ch25h expression was also ob-
served in the PCN-treated cells, but the absolute mRNA
expression of Ch25h in untreated AML12 cells was more

than 50 times lower than that of Cyp3a11 (data not shown).
Troleandomycin tended to upregulate the mRNA expres-
sion of Cyp3a11, but the difference was not statistically sig-
nificant. The addition of desmosterol to cell culture
medium did not affect the induction of Cyp3a11 by PCN.
However, desmosterol seemed to inhibit the induction of
Ch25h by PCN.

Figure 3 shows the effects of PCN, troleandomycin, and
desmosterol on protein levels of CYP3A and CH25H. PCN
increased CYP3A protein level, which was associated with
the upregulated transcription of Cyp3a11 (Fig. 2). How-
ever, although the transcription of Ch25h was also upregu-
atated by the addition of PCN, the protein level of CH25H
was not elevated. In addition, desmosterol did not affect
the expression of cellular CYP3A protein, but CH25H pro-
tein level was obviously decreased by desmosterol treatment.

Intact or boiled aliquots of insect cell microsomes over-
expressing recombinant human CYP3A4 (10 pmol of
P450) were incubated at 37°C for 30 min with 200 μM cho-
lesterol and an NADPH generating system, and the sterol
fraction was derivatized to picolinyl esters by two different
methods. Figures 4A, C represent selected reaction moni-
toring (SRM) of samples that were derivatized at 80°C for

min. This derivatizing method generally produced di-
picolinyl esters of oxysterols, and the SRM data indicated
that 25-hydroxycholesterol was a major product of intact
CYP3A4, as well as 4β-hydroxycholesterol. We also deriva-
tized the sample at room temperature for 30 min, which
produced mono-picolinyl ester of 25-hydroxycholesterol
(Fig. 4B, D). The mass spectrum and retention time of
mono-picolinyl 25-hydroxycholesterol are completely dis-
tinct from those of di-picolinyl 25-hydroxycholesterol. The
production of 25-hydroxycholesterol by intact CYP3A4 was
confirmed using this specifi c derivatization technique.

The effects of substrate (cholesterol) concentrations on
various hydroxylase activities in recombinant human
CYP3A4 are presented in Fig. 5. The most signifi cant ac-

ivity was 25-hydroxylation, which was higher than that of
4β-hydroxylation, a marker activity of CYP3A4. Other
hydroxylase activities, i.e., 22R-, 24R-, 24S-, 26-, and
27-hydroxylation were also observed, but the activities were
much lower than that of 4β-hydroxylation. Apparent Vmax
and Km were calculated by Lineweaver-Burk plots. Vmax of
25-, 4β-, 22R-, 24R-, 24S-, 26-, and 27-hydroxylation were
7.0 × 10−4, 2.0 × 10−4, 5.7 × 10−5, 5.8 × 10−5, 3.4 × 10−6, 5.3 ×
10−5, and 2.3 × 10−5 mol/s/mol P450, respectively, and Km of
those hydroxylations were 182, 62, 37, 161, 15, 80, and
45 μM, respectively.

In Table 1, cholesterol 25- and 4β-hydroxylase activities
are compared among four different insect cell microsomes
containing recombinant human CYP1A2, CYP2C9,
CYP2D6, or CYP3A4. Not only CYP3A4 but also the other
three P450 enzymes signifi cantly catalyzed 25-hydroxyla-
tion of cholesterol, but these activities were lower than
that by CYP3A4. In contrast, 4β-hydroxylation of chole-
sterol was exclusively observed in microsomes containing
CYP3A4. Control microsomes without expressed human
P450 enzymes did not convert cholesterol into 25-hydroxy-
cholesterol or 4β-hydroxycholesterol.
The relationships between serum 25-hydroxycholesterol concentrations and serum 4β-, 24S-, and 27-hydroxycholesterol concentrations in 78 normal Japanese subjects are shown in Fig. 6. Serum 25-hydroxycholesterol concentrations correlated significantly with 4β-hydroxycholesterol concentrations (Fig. 6A), but did not correlate significantly with the concentrations of 24S-hydroxycholesterol (Fig. 6B) or 27-hydroxycholesterol (Fig. 6C). On the other hand, serum 24S-hydroxycholesterol and 27-hydroxycholesterol concentrations correlated significantly ($r = 0.408$, $P < 0.0005$, $n = 78$) in the group of normal subjects.

**DISCUSSION**

Our results provide strong evidence that 25-hydroxylation of cholesterol is catalyzed by CYP3A. First, CYP3A induction caused the accumulation of 25-hydroxycholesterol in a cell line derived from mouse liver. The addition of desmosterol downregulated CH25H protein in the cells, but did not reduce the concentration of cellular 25-hydroxycholesterol. Second, the presence of significant cholesterol 25-hydroxylation activity was proven by using recombinant human CYP3A4. Third, 25-hydroxycholesterol concentrations in normal human sera correlated positively with the 4β-hydroxycholesterol level; a known marker of CYP3A4 activity (23, 24).

In this study, we paid close attention to identifying 25-hydroxycholesterol by using two different derivatization methods, i.e., 80°C for 60 min and room temperature for 30 min. The former method synthesizes the usual dipicolinyl derivative of 25-hydroxycholesterol, whereas the latter method produces the mono-picolinyl derivative, because the C-25 position of 25-hydroxycholesterol is resistant to picolinyl ester formation at room temperature (28). The identification of 25-hydroxycholesterol by our conventional HPLC-MS/MS method was confirmed using...
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Therefore, in the recombinant cytochrome P450 experiments, control assays without adding the NADPH generating system were conducted simultaneously and the data were subtracted from those obtained using the complete assay system.

It was surprising that recombinant CYP3A4 produced much more 25-hydroxycholesterol than 4β-hydroxycholesterol, which is used as a marker of CYP3A4 activity (23, 24). However, serum concentrations of 25-hydroxycholesterol were low compared with those of 4β-hydroxycholesterol (Fig. 6A), which may be explained by the fact that the metabolism of 25-hydroxycholesterol is faster than that of 4β-hydroxycholesterol (31). Whereas 4β-hydroxycholesterol is metabolized slowly by CYP7A1 and CYP27A1 (31), 25-hydroxycholesterol is metabolized faster by CYP7A1 (32) and CYP7B1 (33).

It has been reported that 25-hydroxycholesterol is synthesized not only by CH25H (13) but also by CYP27A1 (16, 17) and CYP46A1 (18). Because only very low levels of this specific derivatization technique. Furthermore, we quantified 25-hydroxycholesterol with great care because this oxysterol may be a normal contaminant of the substrate (cholesterol) and could be generated by cholesterol autoxidation.

Fig. 3. Effects of PCN, troleandomycin (TAM), and desmosterol (DES) on CYP3A and CH25H protein in AML12 cells. Cells were incubated with PCN (10 μM), TAM (100 μM), DES (30 μM), or DES (30 μM) plus PCN (10 μM) for 72 h. Cell homogenates (10 μg protein per lane) were subjected to SDS-PAGE analysis.

Fig. 4. SRM chromatograms obtained during HPLC-ESI-MS/MS analysis of the oxysterol fraction from an incubation mixture of overexpressed recombinant human CYP3A4 (A, B) or boiled CYP3A4 (C, D) with 200 μM cholesterol. The oxysterol fraction was derivatized to picolinyl esters by two different methods, 80°C for 60 min (A, C) and room temperature for 30 min (B, D). The former produces di-picolinyl esters of 25-hydroxycholesterol (25HC) and 4β-hydroxycholesterol (4βHC), whereas the latter produces the mono-picolinyl ester of 25HC. 25HC-d3 (1 ng) and 4βHC-d7 (5 ng) were added to each incubated mixture as internal standards. The same Hypersil GOLD column and the same mobile phase were used for HPLC separation of both di- and mono-picolinyl esters of 25HC. The numbers on the right upper side of each chromatogram represent the full scale of the chromatogram.
25-Hydroxylation, because cholesterol 4β-hydroxylation can be catalyzed by CYP1A2, CYP2C9, and CYP2D6. While CYP3A4 is the most abundantly expressed form of P450 in human liver, cholesterol 4β-hydroxylation can be catalyzed by CYP1A2, CYP2C9, and CYP2D6. The results lend support to the hypothesis that CYP3A4 catalyzes 25-hydroxylation of cholesterol. These results suggest that 25-hydroxycholesterol may be relatively important in humans.

Our results showed that not only CYP3A4 but also CYP1A2, CYP2C9, and CYP2D6 catalyzed 25-hydroxylation of cholesterol to some extent (Table 1). However, CYP3A4 is the most abundantly expressed form of P450 in human liver (as much as 60% of all hepatic P450) (34). In addition, because cholesterol 4β-hydroxylase activities by CYP1A2, CYP2C9, and CYP2D6 were negligible, the positive correlation between serum concentrations of 25-hydroxycholesterol and 4β-hydroxycholesterol cannot be explained by these P450 activities. Thus, at least in normal human subjects, most of the serum 25-hydroxycholesterol appears to originate from CYP3A4.

Under abnormal conditions, however, serum 25-hydroxycholesterol concentrations may not change with 4β-hydroxycholesterol levels. For example, in a patient with cerebrotendinous xanthomatosis (CTX), CYP27A1 deficiency, serum 25-hydroxycholesterol concentration was low but 4β-hydroxycholesterol concentration was high compared with those in a normal subject (28). Because CYP3A4 activity is not significantly altered in CTX (21), it is likely that these oxysterol concentrations were affected by the activities of other enzymes, i.e., impaired CYP27A1 and upregulated CYP7A1 (21) that metabolize 4β-hydroxycholesterol and 25-hydroxycholesterol, respectively. A recent report by Diczfalusy et al. (15) showed that intravenous injection of lipopolysaccharide (endotoxin) in healthy volunteers resulted in an increase in plasma 25-hydroxycholesterol concentration. Although CH25H activity was not determined in these subjects, the increase might be due to the induction of CH25H, as suggested by their experiments using mouse macrophage.

The biochemical role of the production of 25-hydroxycholesterol by CYP3A remains unclear. However, this oxysterol appears to be further metabolized to bile acids (35), which may be one of the important alternative pathways for bile acid biosynthesis. In addition, this oxysterol is a potent inhibitor of HMG-CoA reductase and a ligand of LXRα, so that it may participate in the regulation of lipid metabolism. It should be noted that CYP3A4 catalyzes not only 25-hydroxylation but also 4β-hydroxylation, 22R-hydroxylation, and other nonsterospecific hydroxylations of cholesterol, including 24R-, 24S-, 26-, and 27-hydroxylation (Fig. 5). Because 4β-hydroxycholesterol, 22R-hydroxycholesterol, and 24S-hydroxycholesterol have been reported to be more potent activators of LXRα compared with 25-hydroxycholesterol (8, 9), the influence of CYP3A induction on LXRα activity is not explained by the effects of 25-hydroxycholesterol alone.

Fatty liver and hypertriglyceridemia are characteristic features in Cyp27b1−/− mice (36) but not in CTX patients. Because CYP3A is markedly upregulated in Cyp27b1−/− mice but not in CTX patients (21), oxysterols synthesized by CYP3A may induce fatty liver in Cyp27b1−/− mice. In fact, SREBP1, a target gene of LXRα, and SREBP1-regulated FA biosynthetic enzymes were upregulated in Cyp27b1−/− mice (36), whereas SREBP1 was not upregulated in CTX patients (37).

### TABLE 1. Cholesterol 25- and 4β-hydroxylation activities in recombinant overexpressed human cytochrome P450 (baculosomes)

<table>
<thead>
<tr>
<th>Baculosomes</th>
<th>P450 concentration (pmol P450/mg protein)</th>
<th>25-Hydroxylation (pmol/min/mg protein)</th>
<th>4β-Hydroxylation (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT control</td>
<td>0</td>
<td>0.06 (0.07, 0.05)</td>
<td>0.01 (0.00, 0.01)</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>98</td>
<td>0.58 (0.54, 0.62)</td>
<td>0.12 (0.13, 0.11)</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>313</td>
<td>1.36 (1.50, 1.21)</td>
<td>0.25 (0.28, 0.21)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>292</td>
<td>0.59 (0.64, 0.54)</td>
<td>0.14 (0.17, 0.10)</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>96</td>
<td>1.86 (2.07, 1.64)</td>
<td>0.99 (1.08, 0.89)</td>
</tr>
</tbody>
</table>

**Notes:**
- WT, wild type.
- *Microsomes prepared from insect cells that were infected with baculovirus containing the cDNAs for human cytochrome P450 and rabbit cytochrome P450 reductase.
- **Average of two assays. Individual values in parentheses.
- Control microsomes prepared from insect cells that were infected with a wild-type baculovirus.
In summary, 25-hydroxycholesterol was quantified using the latest HPLC-ESI-MS/MS technique in a mouse liver cell line, in microsomes overexpressing recombinant human cytochrome P450 enzymes and in normal human sera. All data support the idea that CYP3A was one of the responsible enzymes that catalyzed the 25-hydroxylation of cholesterol.

REFERENCES


